



## **Comparative studies of genotoxicity and anti-plasmodial activities of stem and leaf extracts of *Alstonia boonei* (De Wild) in malaria-infected mice**

**Babamale, O. A.,<sup>1</sup> Iyiola, O. A.,<sup>4</sup> Adeyemi, S. B.,<sup>2</sup> Sulaiman, A. F.,<sup>3</sup> Abdulkareem, A. O.,<sup>5</sup> Anifowoshe, A. T.,<sup>4</sup> Awe, O. D.,<sup>1</sup> Ajani, D.<sup>1</sup> and Ugbomoiko, U. S.<sup>1</sup>**

<sup>1</sup>Department of Zoology (Parasitology Unit), University of Ilorin, Ilorin

<sup>2</sup>Department of Plant Biology, University of Ilorin, Ilorin

<sup>3</sup>Department of Biochemistry, University of Ilorin, Ilorin

<sup>4</sup>Department of Zoology (Cell Biology and Genetics Unit), University of Ilorin, Ilorin

<sup>5</sup>Department of Zoology (Animal Physiology Unit), University of Ilorin, Ilorin

email: olas4nice2004@yahoo.co.uk

### **Abstract**

Drug resistance in malaria infection is a serious public health challenge. Thus, scientific search for alternative treatment measures among the local medicinal plants is exigent. We therefore investigated the anti-plasmodial efficacy and genotoxicity of the methanolic leaf and stem extracts of *Alstonia* plant at varying concentration (200 mg/kg, 400 mg/kg and 600 mg/kg) in mice infected with chloroquine sensitive *Plasmodium berghei*. The phytochemical screening of the extract revealed that leaf sample contained significantly higher secondary metabolites, except saponins ( $p < 0.05$ ). Anti-plasmodial activities of the two extracts were duration and dose- dependent. Stem bark extract showed higher curative potential with inhibition rate of 56.71% at 400 mg/kg whereas, leaf extract was efficient at 600mg/kg with 52.15% inhibition rate. Stem bark extract at 400 mg/kg improved the enzymatic activities of the mice; it lowered serum ALT ( $6.88 \pm 4.42$ ) and increased liver ALT ( $41.07 \pm 5.56$ ). Similarly, 400 mg/kg leaf extract showed highest AST ( $70.65 \pm 4.00$ ) and ALT ( $44.65 \pm 7.83$ ) activities in the kidney and liver respectively. Analysis of genotoxicity revealed that micronucleus and abnormal (binucleated, notched and blebbed) were prevalent among the experimental mice which increased significantly ( $p < 0.05$ ) at all concentrations except at 600mg/kg leaf extract. Therefore, this present study indicates that both leaf and stem bark extracts of *A. boonei* possess anti-plasmodial activity and are less genotoxic when compared with standard drug.

**Keywords:** Genotoxicity; *Plasmodium berghei*; metabolites; micronucleus; blebbed; standard drug.

### **Introduction**

Malaria is a global health problem, more than one billion people are currently at risk of infection with thousands of deaths, occurring annually particularly among pregnant women and under five-year children [1]. *Plasmodium falciparum* has been consistently reported as the commonest species of human malaria parasites, accounting for the majority of malaria related morbidity and mortality in most endemic regions of Africa [2]. In Nigeria, infection among less immuned individuals is characterized with severe anemia, cerebral malaria, acute respiratory distress, clinical jaundice, organ congestion, sinusoidal blockage, cellular inflammation and hepatosplenomegaly [3].

Despite the substantial progress made in the treatment

of parasitic diseases globally, rapid development of resistance has made malaria a significant therapeutic challenge infection worldwide. Previous reports have ascribed reduced sensitivity to ACTs and parasites recrudescence to malaria treatment failure [4, 5]. Therefore, the need for continuous scientific search for new drugs with novel modes of action in parasite clearance, restoration of damaged organs/cells during malaria pathogenesis and less toxicity is exigent [6].

In Africa, *A. boonei* is one of the widely used plants in the treatment of fever- related diseases [7]. The plant belongs to the family Apocynaceae found in the continents of Africa, Asia and America [8]. In Nigeria, the plant has been documented to be useful in the treatment of many ailments such as fever, rheumatic

pains, chronic diarrhea, and insomnia [9]. The presence of flavonoid, alkaloid, tannin, saponin and steroids, as well as minerals (calcium, magnesium, sodium, potassium, phosphorus and iron) and Vitamin C in both aqueous and alcoholic extracts of *A. boonei* has been presumed as the major player in the medicinal value of the plant [10].

Anti-plasmodial activities of ethanolic extracts of stem bark and leaf of *A. boonei* had been previously and separately documented by many authors [11, 12]. However, investigations on the effects of this plant on physiological and biochemical components of human system are largely lacking in literature. This study was therefore designed to assess the comparative genotoxicity and anti-malaria activities of methanolic extracts of stem and leaf of *A. boonei*, with the aim of finding rich source of new drugs with less or no toxicity.

## Materials and methods

### Collection of plant materials

Freshly harvested leaves and stem barks of *A. boonei* were collected from a forest in Esa Oke, Osun State, Nigeria (7° 46' 0" North, 4° 54' 0" East). The samples were authenticated at the Department of Plant Biology Herbarium, University of Ilorin (Voucher No. UIH001/1035). The plant samples were thoroughly washed and air dried at room temperature for three weeks. Samples were powdered and stored in air-tight containers at 4°C for further use.

### Preparation of plant extracts and phytochemical screening

Extraction was done using the modified method of Adeyemi *et al* [13]. Five hundred grannes each of the powdered leaves and stem bark were weighed and macerated in 2,500 ml of 70% methanol for 72 hours with intermittent stirring using spatula. The mixtures were filtered using Whatman No. 1 filter paper and concentrated to dryness using rotary evaporator. The crude extracts were dispensed into labelled sample vials and stored at 4°C prior to further use. The extracts were subsequently analyzed for the presence and quantity of secondary metabolites such as tannins, saponins, alkaloids flavonoids, steroids, terpenes and phenols using the methods described by AOAC [14].

### Experimental animals

Fifty-five Swiss albino mice, *Mus musculus* weighing between 14-23 g were purchased from the animal house of Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna, Kaduna State. All animals were housed in plastic cages with net roofing and saw dust beddings and kept in the animal house, University of Ilorin, Ilorin, and were maintained under standard laboratory conditions with free access to standard Recommended Dietary Allowance (RDA) feed (pellet) and water *ad libitum*. They were allowed to acclimatize for 14 days. The study was approved by University of Ilorin Ethical Review Committee (UERC) (approval number: UERC/ASN/2016/513).

### Inoculation of parasites and estimation of parasitaemia

A chloroquine-sensitive *P. berghei* (ANKA strain) was obtained from the animal house at National Institute of Medical Research (NIMR), Lagos State, and 1 ml of blood was taken from donor mice and diluted with normal saline; such that 0.2 ml of the infected-blood contained inoculums of  $1 \times 10^7$  infected red blood cells. The mice were screened for malaria parasites in their blood and only those that were free from malarial parasites were selected for this study. The parasite was then allowed to incubate in the mice for 72 hours after which thin blood film was prepared from caudal tip, and stained using Giemsa stain and viewed under x100 objective of the light microscope. Estimation of the parasitaemia was done according to Babamale and Ugboimo [15].

### Animal grouping for infection and treatment

The fifty-five acclimatized mice were inoculated intraperitoneally except untreated group (Group A). The mice were randomly divided into eleven groups with five mice each. Group B (untreated infected group) was inoculated with *P. berghei* and received no treatment. Group C which served as standard control was inoculated with *P. berghei* and treated with standard drug, chloroquine at 5 mg/kg body weight (b.w). Groups D and E (prophylactic groups) were treated with 400 mg/kg b.w each of leaf and stem bark extracts of *A. boonei* respectively, for eight days at a day interval before parasite inoculation. Groups F, G, H, I, J and K served as curative groups and were inoculated before treatment. Groups F, G and H were treated with the leaf extract of *A. boonei* at 200 mg/kg, 400 mg/kg and 600 mg/kg respectively, while Groups I, J and K were treated with stem bark extract of *A. boonei* at 200 mg/kg, 400 mg/kg and 600 mg/kg respectively. The animals were administered with the extract 2 hours after the parasite was inoculated on day 0 (DO).

### Determination of liver, heart and kidney function test

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using Randox diagnostic kits which are products of Randox Laboratory Limited, Antrim, United Kingdom. Activities of ALT in liver and AST in heart and kidney were also determined by the same kit.

### Micronucleus assay

After 24 hours of the last treatment, thin smear was prepared according to [16]. The films were allowed to air-dry overnight in a dust free environment, fixed in absolute methanol for 20 minutes. The fixed smears were again stained in 10% May-Grunwald (rinse and dry overnight) and 5% Giemsa stains (rinse and dry over-night) after which the stained slides were rinsed with distilled water to remove excess stain and allowed to dry.

### Statistical analysis

All numerical data were subjected to statistical analysis

using the Statistical Package for Social Sciences (SPSS) 16.0. The group mean  $\pm$  S.E.M were calculated for each data and significant differences between the groups were evaluated using one way analysis of variance (ANOVA). Post-test analyses were done using the Duncan Multiple Range at 99.5% significance level ( $\alpha = 0.05$ ).

## Results

The phytochemical screening of the leaf and stem bark extracts of *A. boonei* revealed the presence alkaloids, anthraquinones, flavonoids, phenols, saponins, steroids, tannins and terpenoids, while cardiac glycosides were absent in both samples (Table 1). The leaf sample of the plant contained significantly higher screened secondary metabolites, except saponins ( $p < 0.05$ ). The prophylactic and curative properties of the extracts on *P. berghei* were comparable as detailed in Table 2. There was a daily increment in parasitaemia level among

untreated infected mice while both chloroquine and extracts treated groups recorded daily reduction. Chloroquine demonstrated the highest activity with percentage inhibition of 64.14% and 17.80 days mean survival time. However, anti-plasmodial activities of the two extracts were duration and dose dependent. Stem bark extract showed higher curative potential with inhibition rate of 56.71% at 400 mg/kg whereas, leaf extract was efficient at dose of 600 mg/kg with 52.15% inhibition rate.

The results of enzymatic activities across different groups of the experimental mice are presented in Table 3. The untreated infected mice showed significantly higher activities of ALT ( $16.70 \pm 2.91$ ) and AST ( $28.48 \pm 2.76$ ) in the serum and lower ALT in the liver ( $19.02 \pm 5.18$ ), as well as lower AST in the heart ( $35.22 \pm 1.23$ ) and kidney ( $43.05 \pm 23.37$ ), as compared to uninfected. Oral administration of chloroquine and

**Table 1.** Phytochemical composition of *A. boonei* leaves and stem.

| S/No | Phytochemicals               | Leaves                            | Stembarks                       |
|------|------------------------------|-----------------------------------|---------------------------------|
| 1    | Alkaloids (Mg/100g)          | *448.33 $\pm$ 6.01 <sup>a</sup>   | 350.00 $\pm$ 12.58 <sup>b</sup> |
| 2    | Anthraquinones (Mg/100g)     | 68.33 $\pm$ 4.41 <sup>a</sup>     | 31.67 $\pm$ 1.67 <sup>b</sup>   |
| 3    | Cardiac Glycosides (Mg/100g) | 0.00 $\pm$ 0.00 <sup>a</sup>      | 0.00 $\pm$ 0.00 <sup>a</sup>    |
| 4    | Flavonoids (Mg/100g)         | 761.67 $\pm$ 7.26 <sup>a</sup>    | 626.67 $\pm$ 7.26 <sup>b</sup>  |
| 5    | Phenols (GAE/g)              | 39.63 $\pm$ 0.09 <sup>a</sup>     | 23.23 $\pm$ 0.15 <sup>b</sup>   |
| 6    | Saponins (Mg/100g)           | 43.33 $\pm$ 4.41 <sup>a</sup>     | 86.67 $\pm$ 6.01 <sup>b</sup>   |
| 7    | Steroids (Mg/100g)           | 331.67 $\pm$ 7.26 <sup>a</sup>    | 245.00 $\pm$ 8.66 <sup>b</sup>  |
| 8    | Tannins (Mg/100g)            | 670.00 $\pm$ 7.63 <sup>a</sup>    | 121.67 $\pm$ 7.26 <sup>b</sup>  |
| 9    | Terpenoids (Mg/100g)         | 1,360.00 $\pm$ 10.41 <sup>a</sup> | 223.33 $\pm$ 11.67 <sup>b</sup> |

\*Results are presented as Mean  $\pm$  SEM; data with the same superscript are not significant ( $p > 0.05$ ).

**Table 2.** Comparison of anti-plasmodial activities, inhibition rate and survival time of the methanolic extracts of *Astonia boonei*.

| Variables                         | Day 2                            | Day 4                            | Day 6                             | Day 8                             | %Inhibition       | Mean Survival Time |
|-----------------------------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|-------------------|--------------------|
| <b>Control groups</b>             |                                  |                                  |                                   |                                   |                   |                    |
| A (positive group)                | 0.00 $\pm$ 0.00 <sup>a</sup>     | 0.00 $\pm$ 0.00 <sup>a</sup>     | 0.00 $\pm$ 0.00 <sup>a</sup>      | 0.00 $\pm$ 0.00 <sup>a</sup>      | 100 $\pm$ 0.00    | 18.00 $\pm$ 0.00   |
| B (negative group)                | 476.69 $\pm$ 171.52 <sup>b</sup> | 811.28 $\pm$ 188.27 <sup>d</sup> | 808.21 $\pm$ 108.70 <sup>c</sup>  | 824.87 $\pm$ 192.38 <sup>c</sup>  | 0 $\pm$ 0.00      | 7.20 $\pm$ 1.79    |
| C (standard group)                | 421.16 $\pm$ 139.75 <sup>b</sup> | 204.33 $\pm$ 83.34 <sup>b</sup>  | 136.16 $\pm$ 51.72 <sup>a</sup>   | 109.29 $\pm$ 57.20 <sup>ab</sup>  | 64.14 $\pm$ 35.34 | 17.80 $\pm$ 0.45   |
| <b>Prophylactic groups</b>        |                                  |                                  |                                   |                                   |                   |                    |
| D (400mg/kg <i>Alstonia</i> leaf) | 489.99 $\pm$ 159.98 <sup>b</sup> | 502.92 $\pm$ 102.99 <sup>c</sup> | 437.54 $\pm$ 180.39 <sup>b</sup>  | 433.72 $\pm$ 159.94 <sup>d</sup>  | 32.13 $\pm$ 23.63 | 10.00 $\pm$ 0.71   |
| E (400mg/kg <i>Alstonia</i> stem) | 692.22 $\pm$ 444.42 <sup>b</sup> | 569.30 $\pm$ 350.16 <sup>b</sup> | 465.36 $\pm$ 284.75 <sup>c</sup>  | 321.23 $\pm$ 312.69 <sup>b</sup>  | 22.11 $\pm$ 46.58 | 15.20 $\pm$ 0.83   |
| <b>Treatment groups</b>           |                                  |                                  |                                   |                                   |                   |                    |
| F (200mg/kg <i>Alstonia</i> leaf) | 511.71 $\pm$ 137.40 <sup>b</sup> | 411.17 $\pm$ 132.79 <sup>c</sup> | 337.55 $\pm$ 105.01 <sup>b</sup>  | 291.59 $\pm$ 64.31 <sup>c</sup>   | 41.24 $\pm$ 32.95 | 14.20 $\pm$ 5.76   |
| G (400mg/kg <i>Alstonia</i> leaf) | 553.99 $\pm$ 198.98 <sup>b</sup> | 558.03 $\pm$ 245.09 <sup>c</sup> | 427.87 $\pm$ 65.74 <sup>b</sup>   | 322.65 $\pm$ 118.10 <sup>bc</sup> | 30.85 $\pm$ 33.51 | 15.20 $\pm$ 5.17   |
| H (600mg/kg <i>Alstonia</i> leaf) | 609.67 $\pm$ 150.80 <sup>b</sup> | 594.56 $\pm$ 188.79 <sup>c</sup> | 147.90 $\pm$ 60.45 <sup>a</sup>   | 131.15 $\pm$ 44.64 <sup>ab</sup>  | 52.15 $\pm$ 44.67 | 17.10 $\pm$ 0.55   |
| I (200mg/kg <i>Alstonia</i> stem) | 582.34 $\pm$ 124.99 <sup>b</sup> | 553.42 $\pm$ 296.68 <sup>b</sup> | 407.77 $\pm$ 170.34 <sup>c</sup>  | 178.72 $\pm$ 115.41 <sup>b</sup>  | 41.42 $\pm$ 38.29 | 15.40 $\pm$ 1.52   |
| J (400mg/kg <i>Alstonia</i> stem) | 724.29 $\pm$ 274.08 <sup>b</sup> | 573.85 $\pm$ 163.50 <sup>b</sup> | 362.50 $\pm$ 114.33 <sup>bc</sup> | 124.47 $\pm$ 113.66 <sup>ab</sup> | 56.71 $\pm$ 55.49 | 17.40 $\pm$ 0.90   |
| K (600mg/kg <i>Alstonia</i> stem) | 729.44 $\pm$ 253.08 <sup>b</sup> | 592.90 $\pm$ 284.45 <sup>b</sup> | 508.16 $\pm$ 298.53 <sup>c</sup>  | 225.35 $\pm$ 285.78 <sup>b</sup>  | 28.01 $\pm$ 49.27 | 13.60 $\pm$ 1.95   |

\*Different superscripts across the row depicts significant difference ( $p < 0.05$ ).

methanolic extracts of stem bark and leaf of *A. boonei* significantly reduced the serum levels of ALT and AST and increased the activities of the two enzymes in the tested organs. Stem bark extract at 400 mg/kg and 600 mg/kg significantly increased serum level of AST. In general, stem bark extract at 400 mg/kg showed the lowest value of serum ALT ( $6.88 \pm 4.42$ ) and the highest value of liver ALT ( $41.07 \pm 5.56$ ). Lowest serum AST activity ( $6.31 \pm 2.16$ ) was recorded in 200 mg/kg stem bark extract. Similarly, 400 mg/kg leaf extract showed highest AST ( $70.65 \pm 4.00$ ) and ALT ( $44.65 \pm 7.83$ ) activities in the kidney and liver respectively, while the

serum levels of AST and ALT were not significantly different across the groups treated with leaf extracts at investigated doses.

Table 4 reveals that micronucleus (MN), and binucleated, notched and blebbed nuclear abnormalities are the most nuclear disorders prevalent among the experimental mice. The uninfected group presented no MN while the highest number of MN was observed in standard group. The number of MN in the treated mice increased significantly ( $p < 0.05$ ) at all concentrations except at 600 mg/kg leaf extract when compared with the uninfected-group.

**Table 3.** Effects of extracts'administration enzymatic activities of serum and organs (liver, kidney and heart).

| College                           | Alanine Aminotransferase(ALT) |                        | Aspartate Aminotransferase (AST) |                        |                        |
|-----------------------------------|-------------------------------|------------------------|----------------------------------|------------------------|------------------------|
|                                   | Serum                         | Liver                  | Serum                            | Heart                  | Kidney                 |
| <b>Control groups</b>             |                               |                        |                                  |                        |                        |
| A (uninfected group)              | $6.04 \pm 2.27^a$             | $42.33 \pm 2.52^a$     | $13.26 \pm 1.23^{ab}$            | $46.74 \pm 3.16^b$     | $67.61 \pm 6.46^a$     |
| B (infected untreated group)      | $16.70 \pm 2.91^b$            | $19.02 \pm 5.18^b$     | $28.48 \pm 2.76^c$               | $35.22 \pm 1.23^{ab}$  | $43.05 \pm 23.37^{ab}$ |
| C (standard group)                | $11.34 \pm 2.15^{ab}$         | $35.81 \pm 12.75^{ab}$ | $15.44 \pm 0.93^b$               | $16.74 \pm 2.15^{abc}$ | $50.87 \pm 12.30^{ab}$ |
| <b>Prophylactic groups</b>        |                               |                        |                                  |                        |                        |
| D (400mg/kg <i>Alstonia</i> leaf) | $15.54 \pm 9.34^a$            | $29.65 \pm 6.60^{ab}$  | $25.13 \pm 15.37^b$              | $20.87 \pm 1.84^{ab}$  | $60.90 \pm 0.57^{ab}$  |
| E (400mg/kg <i>Alstonia</i> stem) | $14.55 \pm 3.16^{ab}$         | $33.84 \pm 0.38^{ab}$  | $17.83 \pm 4.31^a$               | $29.57 \pm 2.45^{ab}$  | $59.13 \pm 14.14^{ab}$ |
| <b>Treatment groups</b>           |                               |                        |                                  |                        |                        |
| F (200mg/kg <i>Alstonia</i> leaf) | $15.18 \pm 1.51^a$            | $35.38 \pm 0.23^{ab}$  | $17.17 \pm 4.61^a$               | $30.65 \pm 0.31^{ab}$  | $50.00 \pm 18.44^{ab}$ |
| G (400mg/kg <i>Alstonia</i> leaf) | $11.79 \pm 2.27^a$            | $44.65 \pm 7.83^{ab}$  | $23.26 \pm 20.61^a$              | $10.87 \pm 9.22^a$     | $70.65 \pm 4.00^b$     |
| H (600mg/kg <i>Alstonia</i> leaf) | $14.78 \pm 2.30^a$            | $30.97 \pm 9.81^b$     | $14.79 \pm 1.85^a$               | $10.00 \pm 6.76^a$     | $24.13 \pm 23.67^a$    |
| I (200mg/kg <i>Alstonia</i> stem) | $7.05 \pm 3.66^a$             | $34.91 \pm 2.90^a$     | $6.31 \pm 2.16^a$                | $18.69 \pm 8.61^{ab}$  | $24.99 \pm 5.23^a$     |
| J (400mg/kg <i>Alstonia</i> stem) | $6.88 \pm 4.42^a$             | $41.07 \pm 5.56^a$     | $27.74 \pm 3.07^c$               | $13.70 \pm 8.30^{ab}$  | $48.48 \pm 11.99^{ab}$ |
| K (600mg/kg <i>Alstonia</i> stem) | $12.95 \pm 5.67^{ab}$         | $35.18 \pm 4.79^{ab}$  | $27.65 \pm 4.30^c$               | $10.44 \pm 6.14^a$     | $25.44 \pm 12.61^a$    |

\*Different superscripts across the row depicts significant difference ( $p < 0.05$ ).

**Table 4.** Frequency of micronucleus and other nuclear abnormalities induced by methanolic extract of leaf and stem bark of *A. boonei*.

| Variables                         | MN | Binucleated | Notched | Lobed | Blebbled | Polynucleated | Total abnormalities | MN Induction (%) | NA Induction (%) | X ± S.E        |
|-----------------------------------|----|-------------|---------|-------|----------|---------------|---------------------|------------------|------------------|----------------|
| Control groups                    |    |             |         |       |          |               |                     |                  |                  |                |
| A (positive group)                | 0  | 0           | 0       | 0     | 1        | 3             | 4                   | 0                | 0.4              | 1.3 ± 0.3333   |
| B (negative group)                | 6  | 0           | 4       | 8     | 0        | 2             | 20                  | 0.6              | 1.4              | 6.7 ± 0.6667*  |
| C (standard group)                | 11 | 11          | 11      | 18    | 11       | 13            | 75                  | 1.1              | 6.4              | 25.0 ± 1.1547* |
| Prophylactic groups               |    |             |         |       |          |               |                     |                  |                  |                |
| D (400mg/kg <i>Alstonia</i> leaf) | 2  | 2           | 1       | 1     | 1        | 1             | 8                   | 0.2              | 0.6              | 2.7 ± 0.3333   |
| E (400mg/kg <i>Alstonia</i> stem) | 1  | 1           | 1       | 4     | 0        | 1             | 8                   | 0.1              | 0.7              | 2.7 ± 0.3333   |
| Treatment groups                  |    |             |         |       |          |               |                     |                  |                  |                |
| F (200mg/kg <i>Alstonia</i> leaf) | 5  | 6           | 3       | 6     | 5        | 8             | 33                  | 0.5              | 2.8              | 11.0 ± 1.7321* |
| G (400mg/kg <i>Alstonia</i> leaf) | 5  | 4           | 2       | 4     | 1        | 4             | 20                  | 0.5              | 1.5              | 6.7 ± 0.6667*  |
| H (600mg/kg <i>Alstonia</i> leaf) | 4  | 2           | 0       | 3     | 2        | 2             | 13                  | 0.4              | 0.9              | 4.3 ± 0.8819   |
| I (200mg/kg <i>Alstonia</i> stem) | 0  | 3           | 3       | 6     | 2        | 3             | 17                  | 0                | 1.7              | 5.7 ± 1.7638*  |
| J (400mg/kg <i>Alstonia</i> stem) | 4  | 3           | 4       | 4     | 4        | 2             | 21                  | 0.4              | 1.7              | 1.0 ± 1.0000*  |
| K (600mg/kg <i>Alstonia</i> stem) | 4  | 4           | 0       | 3     | 4        | 2             | 17                  | 0.4              | 1.3              | 1.2 ± 1.2019*  |

\*Significant at  $p < 0.05$ .

## Discussion

This study reveals that aqueous extracts of leaf and stem bark of *A. boonei* possess considerable anti-malarial effects against *P. berghei* infection in mice as evidenced by the percentage of parasite inhibition obtained in the study. It was observed that the anti-plasmodial activities of the extracts are time and dose dependent with threshold doses of 600 mg/kg and 400 mg/kg of leaf and stem respectively. Parasite suppression activity observed in this study is slightly low compared with the report of Alli *et al* [17] in their *in-vivo* study of anti-plasmodial activities of medicinal plant. The anti-malarial activities demonstrated by these extract could be attributed to their alkaloid, tannins, phenols, saponins and flavonoids constituent as previously reported [18, 19]. Moreover, these constituents can also serve as immuno modulators, antioxidants and inhibitors of haem polymerization [20, 21]; particularly saponins and polyphenols have been associated to anti-anemic activities [22].

Tissue activities of the aspartate and alanine transaminases (AST and ALT) are biomarkers of organs functions. Their elevation in serum therefore suggests tissue damage or/and organ dysfunction [23]. Decreased activities of AST and ALT in the investigated organs of the untreated infected mice obtained in this current study are strong indication of pathological condition of malaria infection occasioned by pre-erythrocytic activities of the sporozoites. This observation is consistent with the reports of Onyesom and Onyemakonor [24] and may be ascribed to significant perturbation of organ's parenchyma and membrane, leading to leakage of organs' enzymes into the circulation.

Oral administration of methanolic extracts of leaf and stem bark of *A. boonei* at tested concentrations (200, 400 and 600 mg/kg) significantly increased the liver activity of Alanine Aminotransferase (ALT), indicating the ability of the extracts to recover malaria-induced liver damage. Our findings agree with the study of Momoh *et al* [25] who reported that, leaf extract of *A. boonei* has positive effect on ALT and AST. However, our finding showed that AST activities in both kidney and heart of infected mice treated with standard drug are lower when compared with uninfected control. Thus indicating the likely side effect of the standard drug used in the study. Meanwhile, the extracts lowered the serum activities of AST at all tested concentrations without corresponding increase in its heart and kidney activities, suggesting that the inability of the extracts to completely ameliorate the inhibitory effect of malaria parasite on the synthesis of AST *in situ*.

Despite the therapeutic potential of this plant, its genotoxicity has still not been evaluated. Therefore, the frequency of micro-nucleated cells was analyzed to assess DNA damage in the genetic material of the exposed mice. In this study, the average number of micro-nucleus (MN) in the treated mice increased significantly ( $p < 0.05$ ) at all concentrations of both extracts when compared with the untreated control. However, nuclear abnormalities recorded among the group of animal treated with standard drugs were higher. Thus confirming that none

of the extract at all levels of tested concentration is genotoxic than the standard drug. This observation is consistently in line with the report of Rivera *et al* [26]. Nevertheless, our result shows that leaf extracts at effective dose of 600mg/kg is relatively less genotoxic than 400 mg/kg threshold dose of stem extract. This was evidenced by the progressive amelioration induced by the leaf extract at the said dose on the genetic damage as previously demonstrated by previous researchers [27-29]. The nuclear abnormalities recorded among untreated infected mice were observed to be gradually ameliorated among the infected mice treated with 600 mg/kg *Alstonia* leaf extract.

## Conclusion

This study indicates that both leaf and stem bark extracts of *A. boonei* possess anti-plasmodial activity, confirming the reports of earlier workers. Both extracts of *A. boonei* have tendency of restoring liver and kidney impairments. While leaf extract is better at preventing malaria, stem bark extract is more efficient in treating malaria and at restoring organ impairments. The numbers of MNi observed indicates that both extract are less toxic when compared with standard drug and can therefore serve as best alternative in the treatment of malaria. Further studies to investigate active compounds of the extracts as well as its mechanism of action are recommended.

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